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Two approaches to improve fertility of subclinical mastitic dairy cows

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ABSTRACT

Mastitis, particularly in its subclinical form, is a widely spread disease that reduces the fertility of lactating cows. A major cause of poor conception risk has been associated with delayed ovulation of a large subgroup of subclinical mastitic cows. This study examined 2 approaches to improve fertility in this subgroup. Subclinical mastitic cows were defined by somatic cell count elevated above a threshold of 150,000 cells/mL of milk determined in all monthly test day samples collected before AI. Uninfected (control) cows were defined by somatic cell count below threshold. In experiment 1, we examined a hormonal approach aimed to correct the timing of ovulation in mastitic cows in which it would otherwise be delayed. The probability of conception of mastitic and uninfected groups following Ovsynch (OVS) and timed AI versus AI following detected estrus (E) was examined (n = 1,553 AI) and analyzed by a multivariable, logistic model statement using the GLIMMIX procedure of SAS. The OVS protocol significantly elevated the probability of conception of mastitic cows to a level similar to that of their uninfected counterparts. Actual mean conception risks for uninfected-E, subclinical-E, uninfected-OVS, and subclinical-OVS groups were 41.8, 26.4, 39.3, and 40.5%, respectively. The OVS protocol did not improve probability of conception in cows diagnosed with uterine disease postpartum. In experiment 2, a management approach aimed to better synchronize timing of ovulation with timing of AI in subclinical mastitic cows was examined. A second AI was added 24 h after the first (routine) AI, following detection of natural estrus. Probability of conception did not differ between subclinical mastitic cows inseminated once or twice. Lack of improvement in conception risk might be related to low preovulatory LH surge in mastitic cows, which is likely to induce not only delayed ovulation but also disruption of oocyte maturation. Thus the OVS protocol can improve fertility of subclinical mastitic cows, probably due to "corrected" timing of ovulation in cows in which it would otherwise be delayed.
Key words: subclinical mastitis, fertility, Ovsynch protocol, delayed ovulation

INTRODUCTION

Mastitis is a major disease that causes economic losses to the dairy industry. These losses have been related to decreased milk yield and quality, and increased veterinary services and culling rate, among others (Losinger, 2005; Halasa et al., 2007). During the last decade, however, an association between decreased fertility and clinical and subclinical mastitis has been recognized (Schrick et al., 2001; Santos et al., 2004; Hertl et al., 2010), causing significant disruption of reproductive performance associated with additional loss to dairy farms. In a large epidemiological study of 287,000 AI cows, we showed a significant decrease in the probability of conception in likely subclinical mastitic cows exhibiting chronic elevation of SCC >150,000 cells/mL of milk (Lavon et al., 2011a). Similar findings showing an association between subclinical mastitis and poor conception were then also reported by Hudson et al. (2012). The fact that between 20 and 40% of cows in commercial dairy herds in several countries have subclinical, long-term, chronic mastitis (Ramírez et al., 2014) emphasizes the importance of studying approaches to improve fertility of subclinical mastitic cows.

Unlike clinical infection and its effects on reproduction, which have been studied quite intensively over the years, the mechanism of subclinical mastitis-induced disruption of fertility is poorly documented. Recent studies have shed some light on this issue. It was found that about 30% of naturally occurring subclinical mastitic cows manifest delayed ovulation (Lavon et al., 2010). An estrus-to-ovulation interval of 56 h on

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average was recorded, compared with a normal interval of 28 h in uninfected cows and the remaining, nonsusceptible, 70% of mastitic cows. Delayed ovulation could interfere with the process of fertilization, subsequently leading to poor conception risk (**CR**; no. of pregnancies per no. of inseminations). In a follow-up study, a similar proportion (one-third) of subclinical cows exhibited reduced estradiol and androstenedione concentrations in the follicular fluid of preovulatory follicles (Lavon et al., 2011b). The latter was associated with reduced mRNA expression of major steroidogenic genes in both theca and granulosa cells (Lavon et al., 2011b). Induction of subclinical mastitis showed a proportion of cows (40%) quite similar to that found in naturally occurring mastitic cows that exhibited reduced follicular estradiol concentrations (Furman et al., 2014).

To the best of our knowledge, studies to improve fertility of mastitic cows, particularly those with widely spread subclinical mastitis, have never been attempted. Based on above studies, improved fertility might be achievable for cows with mastitis-induced delayed ovulation. Better synchronization of the timing of ovulation relative to AI, using a hormonal or management approach, could improve fertilization rate. The objective was to improve conception of subclinical mastitic cows by using the Ovsynch protocol, which induces a normal LH surge at the optimal time relative to timed AI (hormonal approach; experiment 1), or by adding a second AI 24 h after the first, regular AI, to better suit the delayed timing of ovulation (management approach; experiment 2).

MATERIALS AND METHODS

Two independent fertility studies were performed. The experiments were conducted according to the guidelines of the animal care and welfare committee of the Hebrew University.

Experiment 1: Study Design for the Hormonal Approach

Data were collected from 3 commercial herds during 2 periods from 2003 through 2007 (early) and from 2009 through 2011 (late). Overall, 1,553 AI of primiparous and multiparous Holstein cows were included. The SCC analyzed from the monthly milk-test samples at the central laboratories of the Israeli Cattle Breeders Association were used as an indication of mastitis. Composite milk samples were obtained by technicians, preserved by the addition of Bronopol (Advanced Instruments, Norwood, MA) and analyzed in a Milkoscan with Fossomatic Analyzer (Foss Electric, Hillerød, Denmark) for SCC. A SCC threshold of 150,000 cells/mL of milk was set to distinguish between uninfected and infected cows, as previously described (Lavon et al., 2011a). This cutoff was based on a meta-analysis of 21 published studies conducted in different countries (Djabri et al., 2002). Cows with 2 or 3 (depending on duration of calving to first AI interval) monthly milk tests before first AI (first postpartum milk test collected excluded) with SCC elevated above the cutoff line, in all samples collected, were considered to be subclinical mastitic cows (Lavon et al., 2011a). Cows with all samples below 150,000 cells/mL of milk were considered to be uninfected, and those with mixed values (above and below the cutoff) were not included in the study. In the early period of the study, cows were classified as uninfected or with subclinical inflammation retrospectively according to SCC profile. In the late period, SCC information was available online from the Israeli Cattle Breeders Association central laboratories and cows were assigned to uninfected or infected subgroups close to expected date of AI. Cows in each classification were assigned randomly to either AI following detection of estrus (**E**) or timed AI following the Ovsynch (**OVS**) protocol. The OVS protocol consisted of first GnRH injection (200 μg of Gonadorelin i.m., Gonabreed, Parnell Laboratories, Alexandria, Australia) followed by $PGF_{2\alpha}$ 7 d later (500 μg of Cloprostenol, Estroplan, Parnell Laboratories) and a second GnRH injection 60 h later, and timed AI 16 h after GnRH. A small proportion of cows that were detected in estrus and inseminated before the scheduled timed AI were excluded from the analysis. Detection of estrus occurred trice daily before milking by a computerized pedometric system (Afimilk, Afikim, Israel). Pregnancy was confirmed by uterine palpation per rectum 40 to 50 d after AI. Cows that manifested estrus before being checked for pregnancy and those that were not diagnosed as pregnant were recorded as nonpregnant.

A total of 1,165 uninfected and 388 subclinical mastitic cows were included in the study. Accordingly, 4 experimental groups were evaluated: uninfected-E (n = 593), uninfected-OVS (n = 572), subclinical-E (n = 193), and subclinical-OVS (n = 195); the subclinical mastitic group contained 25% of all experimental cows. Cows in the E and OVS groups were assigned alternately to their experimental groups in all herds during early and later periods of the study. Also recorded in the study were lactation number, change in BCS (determined according to Wildman et al., 1982) between calving and peak lactation (cutoff at > 0.5), date of AI (summer or winter), and herd-period. Cyclicity before synchronization and AI, as determined by pedometry (Afimilk), was included in the model. Uterine health status postpartum, which was added to the model, included retained fetal membranes (for more than 24